Plasma Surface Modification of Poly(D,L-Lactic Acid) as a Tool to Enhance Protein Adsorption and the Attachment of Different Cell Types

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Received 2 May 2007; revised 24 October 2007; accepted 13 December 2007
Published online 21 March 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.b.31068

Abstract: We have studied the influence of oxygen radio frequency glow discharge (RfGD) on the surface and bulk properties of poly(D,L-lactic acid) (PDLLA) and the effect of this surface modification on both protein adsorption and bone cell behavior. PDLLA films were characterized before and after plasma surface modification by water contact angle, surface energy, and adhesion tension of water as well as by scanning electron microscopy (SEM), X-ray electron spectroscopy (XPS), and Fourier transform infra-red (FTIR) spectroscopy. RfGD-films showed an increase in hydrophilicity and surface energy when compared with untreated films. Surface morphological changes were observed by SEM. Chemical analysis indicated significant differences in both atomic percentages and oxygen functional group. Protein adsorption was evaluated by combining solute depletion and spectroscopic techniques. Bovine serum albumin (BSA), fibronectin (FN), vitronectin (VN), and fetal bovine serum (FBS) were used in this study. RfGD-treated surfaces adsorbed more BSA and FN from single specie solutions than FBS that is a more complex, multi-specie solution. MG63 osteoblast-like cells and primary cultures of fetal rat calvarial (FRC) cells were used to assess both the effect of RfGD treatment and protein adsorption on cell attachment and proliferation. In the absence of preadsorbed proteins, cells could not distinguish between treated and untreated surfaces, with the exception of MG63 cells cultured for longer periods of time. In contrast, the adsorption of proteins increased the cells' preference for treated surfaces, thus indicating a crucial role for adsorbed proteins in mediating the response of osteogenic cells to the RfGD-treated PDLLA surface. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 87B: 59–66, 2008

Keywords: polylactic acid; plasma surface modification; protein adsorption; cell attachment and proliferation

INTRODUCTION

Polylactic acid (PLA) is a well know biodegradable aliphatic polymer that has been previously explored for several biomedical applications such as bone fixation devices and tissue engineering scaffolds.[1,2] Although it is known to be biocompatible and is widely used clinically, its low wettability and surface energy have been shown to affect cell attachment and proliferation and remain an issue.[3] In

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Contract grant sponsors: Presidential Research Enhancement Fund (PREF), UTHSCSA, San Antonio, TX, USA
Contract grant sponsor: Foundation for Science and Technology (FCT), Portugal; contract grant number: SFRH/BD/11188/2002; and partial funding through FEDER and POCTI programs
Contract grant sponsor: EU funded Project HIPPOCRATES; contract grant number: NMP3-CT-2003-505758
Contract grant sponsor: European NoE EXPERTISSUES; contract grant number: NMP3-CT-2004-500283.

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the field of biomaterials it is well established that the surface characteristics of an implant play a more critical role in determining the biologic response compared with the bulk properties of the biomaterial. Thus a wide range of surface modification methodologies have been explored to achieve desirable surface properties, such as chemistry, wettability, surface energy, and topography.[4,5] Often the goal of surface modification techniques is to generate a physical and/or chemical modification of the outer molecular layer of the surface, while retaining the bulk properties of the material, including mechanical ones.[4] In this context, plasma treatments are frequently used to modify the chemical functionality of nonreactive biomaterial surfaces because they can be applied on a wide range of implant shapes and sizes.[6] Although it is accepted that plasma modification can yield irregular surface chemistries,[7] this technique presents a major advantage while enables the use of a diversity of chemicals and thus the production of a variety of special functional groups on the surface.[8] Plasma surface activation employs gases, such as oxygen, which dissociate and react with the surface, creating additional functional groups that can be recognized as adhesion sites for surrounding cells.[9]

Cell response to a biomaterial surface is considered one of the major factors in determining the biocompatibility of a material because this step affects subsequent cell proliferation and differentiation pathways.[10] With this in mind, a number of investigators have reported using plasma treatment to improve the behavior of anchorage-dependent cells such as osteoblasts[11] and endothelial cells.[12,13] It is known that interfacial reactions occurring when a material contacts a biological environment are modulated by both the surface and the biomolecules, such as proteins, that interact with it. The outcome of these interactions subsequently affect the cellular response.[14,15] In the past, studies have tried to assess the effect of surface properties on the adsorption of proteins with the goal of creating biomimetic surfaces, such as those that mimic extracellular matrix (ECM) properties, to improve surface recognition by cells.[16,17] Along these lines, in the present study we aim to provide insight into the interaction of two different types of osteogenic cells with poly(D,L-lactic acid) surfaces with differences in wettability, surface energy, chemistry, and roughness.

An oxygen-based plasma treatment of PDLLA was used to modify surface properties and alter the adsorption of proteins. Characterization of the surface was performed by measuring the surface contact angle and surface energy, and the use of scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and Fourier transform infra-red spectroscopy (FTIR). The adsorption of various proteins was studied as a function of gas plasma treatment; these included nonadhesive proteins such as bovine serum albumin (BSA),[18–20] adhesive proteins such as fibronectin (FN)[21–24] and vitronectin (VN)[25–27] and the complex protein solution fetal bovine serum (FBS). The influence of these proteins on cell attachment and proliferation was analyzed. Two types of cells were used: MG63 cells as a prototype of an established line of bone-like cells, and fetal rat calvarial (FRC) cells as an example of primary osteoprogenitor cells.

MATERIALS AND METHODS

Materials and Chemicals

Poly[D,L-lactide] (PDLLA) was purchased from Birmingham Polymers, Inc. (USA) and acetone from EM Science (Germany). Glass coverslips were obtained from PGC Scientifics (USA). Ultra pure water for contact angle was purchased from Pierce (USA) and diiodomethane was supplied from Sigma (USA). The proteins used were from bovine sources; serum albumin (BSA), plasma fibronectin (FN), vitronectin (VN) and FBS were obtained from Pierce (USA), Sigma (USA), Calbiochem (USA) and Atlanta Biologicals (USA), respectively. Saline solution was purchased from Baxter (USA) and Bicinchronic Acid (BCA) reagents were obtained from Pierce (USA). The enzyme mixture of 0.2% collagenase/0.05% trypsin and Hank's Balanced Salt Solution (HBSS) were obtained from Sigma (USA). Cell culture media αMEM and Penicillin-Streptomycin Mixture were obtained from Gibco, DMEM from CELLGRO, β-glycerophosphate from Caliochem, L-ascorbic acid from Sigma and FBS from Atlanta Biomedicals (USA). WST-1 (TAKARA, Japan) and trypsin-EDTA (Sigma, USA) were used for cell number quantification.

Poly(D,L-Lactic Acid) Films Fabrication

Poly[D,L-Lactide] (PDLLA) films were fabricated under clean conditions using acetone as solvent. The polymer used had an inherent viscosity of 0.67 dL/g and was dissolved in acetone at a ratio of about 1:23 or 1.64 g/37 mL. The polymer solution was cast directly over 1.5 cm diameter glass coverslips. In this fashion, after solvent evaporation, circular PDLLA film samples attached to the glass coverslips were obtained. These were kept under vacuum for further drying until use. The films were sterilized by UV radiation (8 h) in a tissue culture hood prior to use.

Plasma Treatment and Surface Sterilization

The PDLLA film sample surfaces were modified by means of O_2 gas plasma in a Radio Frequency Glow Discharge (RfGD) chamber (Harrick Scientific Corporation, USA). The plasma reactor chamber was stabilized under vacuum to ~26.7 Pa and then O_2 was injected into chamber at a pressure of 15 psi for 30 s followed by a waiting period of 30 s before plasma treatment. Plasma treatment was initiated for 180 s using a power of 100 W and pulsed frequency of 13.5 MHz. Time-related changes on treated surfaces were minimized by testing the samples within the following 48 h. Sterilization of plasma modified PDLLA was performed as described for original surfaces.

Characterization of PDLLA Surfaces

Water Contact Angle. The relative hydrophilicity of gas plasma treated and nontreated PDLLA surfaces was assessed by water contact angle measurements. Contact angles were measured using the sessile drop method on a Video Contact Angle 2000 System (AST Products, USA) and ultra-pure water. Both side of the water drops were recorded and averaged; nine drops and three samples per condition were used and the results averaged. Measurements were recorded 10 s after liquid contact with the surface.

Contact angle measurements were used to investigate the wettability of the surfaces following both RfGD modification and UV sterilization experimental steps.

Surface Energy and Adhesion Tension of Water. Surface energy measurements on control and plasma treated PDLLA films were performed in accordance with the Owens and Went method[28] that distinguishes between polar (γ^{p}) and disperse or nonpolar (γ^{d}) components of the surface energy. Literature sources report surface tension values for water and diiodomethane as 72.8 and 50.8 dyne/cm at 20°C, respectively.[29] Moreover, polar and disperse parts for water were considered to be 51.0 dyne/cm and 21.8 dyne/cm and for diiodomethane 0.0 dyne/cm and 50.8 dyne/cm, respectively.[29]

Water adhesion tension was determined by multiplying the contact angle ϑ of water on the surfaces by the surface tension γ_1 of water (72.8 m*N*/m).[29] Thus, both the adhesion tension of water and surface energy determinations were based on the sessile drop method.

X-Ray Photoelectron Spectroscopy. X-ray photoelectron spectroscopy (XPS) measurements were performed before and following plasma treatment in order to determine the surface composition of the PDLLA films. Measurements were taken at five different points on each surface. These experiments were carried out using a Kratos Axis-Ultra (Kratos Analytical, UK) with monochromatic Al X-ray source. The X-ray energy was 1486.6 eV and the base pressure was $\sim 2.9 \times 10^{-11}$ Pa.

Fourier Transform Infra-red Spectroscopy. FTIR spectra were obtained in the attenuated total reflection (ATR) mode using a Nicolet Spectrometer (Nicolet Instrument Coorporation, USA). Each spectrum was recorded with a total of 32 scans and 4.0 cm^{-1} resolution after 20 s of vacuum chamber stabilization. Original and treated surfaces were analyzed in triplicates in the range 400–4000 cm^{-1}.

Scanning Electron Microscopy. Samples were sputter coated (Med-010 Sputter Coater by Balzers-Union, USA) with a thin Au-Pd layer and examination was performed using a scanning electron microscope (Leica, UK). Triplicates were prepared for all control and plasma treated PDLLA surfaces.

Protein Adsorption Assay

Two different protein adsorption studies were performed. In both cases, glass coverslips were used as control surfaces. Single and complex protein solutions were prepared for incubation with PDLLA surfaces before and after plasma treatment with the goal to assess the effect on individual molecules and to mimic more complex protein environments.

For the first protein adsorption study the following solutions were prepared: 1000 μg/mL of BSA, 100 μg/mL of fibronectin (FN), 0.7 μg/mL of vitronectin (VN) and 1000 μg/mL of FBS. Proteins and controls were individually incubated with characterized samples for 15 min at 37°C as described elsewhere.[30] Protein adsorption for BSA and FN was assessed by coupling a depletion method with a protein assay as follows: after incubation, the amount of nonadsorbed protein in the solution was quantified using the Micro-BCA assay and reading visible emission (570 nm) in a Micro Plate Reader (BIO-RAD, USA). Glass coverslips were used as control surfaces and control polymer surfaces were prepared using incubation in saline solution. Unintended protein loss from other sources was controlled by means of using positive displacement pipettes, capillaries and pistons purchased from Gilson Medical Electronics S. A. (France).

In a second set of experiments, protein solutions were prepared at 1% of the concentration of those proteins in human blood plasma[18]: 350 μg/mL of BSA, 4 μg/mL of FN, 3 μg/mL of VN and 1% (V/V) of FBS. As previously stated, protein incubation followed a method described elsewhere.[30]

Attachment and Proliferation of FRC and MG63 Cells

Cell seeding was performed immediately after the protein adsorption step to avoid surface drying and consequent protein conformational changes or denaturation. Surface rinsing was not performed and any enrichment of the cell culture media that could result from remaining nonadherent proteins were assumed negligible.

Primary Cell Culture. Fetal rat calvaria (FRC) cells were isolated by sequential enzyme digestions from calvaria of 21 days Sprague-Dawley rat fetuses as described elsewhere.[31] Briefly, calvaria (frontal and parietal bones) were aseptically removed and stripped of the periosteum. The minced fragments underwent nine sequential digestions in fresh 0.2% collagenase/0.05% trypsin in Hank's Balanced Salt Solution (HBSS) for 20 min at 37°C. Cells were resuspended in α-MEM enriched with 10% FBS, plated in T-75 falcon tissue culture flasks and incubated at 37°C in 5% CO_2 until confluent. Adherent cells were considered viable. For long culture periods, cells were trypsinized and grown as described by Bellows et al.[32] Populations II–V were seeded at 4×10^4 cells/mL in αMEM containing 1 mg/mL of β-glycerophosphate, 0.05 mg/mL of L-ascorbic

acid, and 10% (V/V) FBS onto the different surfaces: PDLLA, plasma treated PDLLA and the same surface batches after carrying out the first protein adsorption procedure described above at: 1000 μg/mL BSA, 100 μg/mL FN, 0.7 μg/mL VN and 1000 μg/mL of FBS. Incubation was performed for 3 h, and 7, 9, and 14 days. Attachment (measured at the 3-h time point) and proliferation (assessed with the 7-, 9-, and 14-day time points) measurements were conducted by trypsinization of the cultures and then cell counting using a Coulter Zi Dual cell counter (Coulter Corporation, USA). Appropriate controls were used, including tissue culture polystyrene (TCPS).

Cell Line Culture. Cell response was also studied using human MG63 osteoblast-like osteosarcoma cells (American Type Culture Collection, Rockville, MD) that have been well characterized in the literature and provide a good model for the study of human bone cells. Cells were incubated for 1, 4, and 7 days in DMEM containing 10% FBS and 1% penicillin-streptomycin mixture. In this study, the plasma protein environment was simulated by preparing protein solutions at 1% of their concentration in human blood plasma: 350 μg/mL of BSA, 4 μg/mL of FN, 3 μg/mL of VN and 1% (V/V) of FBS.[18] The films were seeded at 4×10^4 cells/mL and cell proliferation was assessed by WST-1 assay. After each incubation period, cultured samples were transferred to new wells with fresh media and analyzed for mitochondrial activity using the colorimetric WST-1 tetrazolium conversion assay (TAKARA, Japan). Briefly, 10 μL of WST-1 reagent was added per well, and the cells were incubated for an additional 2 h. The absorbance of the WST-1-containing cell supernatant was determined at 450 nm (Benchmark Microplate Reader, Bio-Rad, USA). To avoid interference from both cell culture media and PDLLA surfaces, the following controls were prepared and considered as blank samples: fresh media and PDLLA samples immersed in fresh media but with no cells seeded.

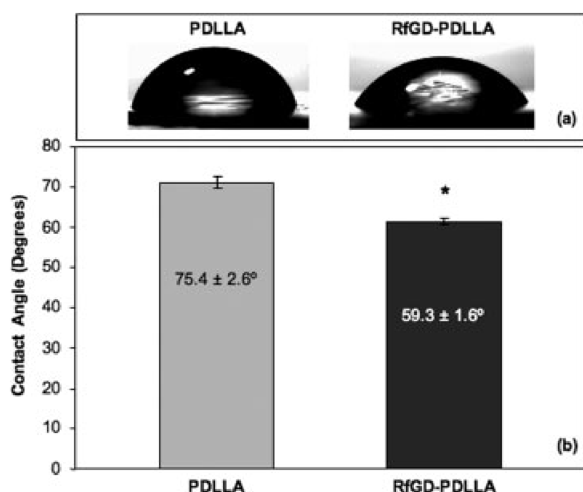

Figure 1. Water drop profiles (a) and contact angle measurements (b) for PDLLA and plasma treated PDLLA films. *Statistically different from PDLLA (t test; Bi-tail; $p < 0.05$; $n > 9$).

TABLE I. Adhesion Tension of Water and Surface Energy Measurements for Nontreated and RfGD-PDLLA

	Surface Energy (dyne/cm)	Adhesion Tension (mN/m)
PDLLA	42.7 ± 2.3	18.3 ± 3.2
RfGD-PDLLA	50.0 ± 1.8[a]	37.2 ± 1.8[a]

[a] Statistically different from PDLLA (t test; Bi-tail; $p < 0.05$; $n > 9$).

Statistical Analysis

Results of the tests were tabulated as mean ± SD. The effects of plasma treatment on both the surface parameters and cell density values were statisticly analyzed by two-tail Students t test and differences were considered significant at $p < 0.05$.

RESULTS

Characterization of PDLLA and Plasma-Treated PDLLA Films

The effect of oxygen based RfGD treatment on PDLLA films was assessed by several surface analytical techniques: water contact angle, surface energy, adhesion tension of water, SEM, XPS and FTIR-ATR.

In Figure 1, surface wettability obtained by the sessile drop method is presented as a function of plasma treatment. Contact angle measurements are frequently used because of their sensitivity and because they allow for studying surface changes within the top few atomic layers.[33] As seen in Figure 1(a), the characteristic water drop profiles show lower spreading for the control PDLLA surfaces when compared with plasma treated surfaces. As a consequence of the oxygen-based plasma surface modification, contact angles decreased from 75.4° ± 2.6° to 59.3° ± 1.6° [Figure 1(b)], which comprises a hysteresis of 6°. Thus the results indicate an increase in the hydrophilicity of the RfGD-PDLLA surface ($p < 0.05$). Contact angle measurements were also performed after UV sterilization on both treated and control samples because UV radiation is a physico-chemical modification methodology frequently used for photo-activation of polymers and could possibly alter the surface properties.[4] However, measurements of contact angles obtained before and after sterilization by UV do not reflect any statistical differences between PDLLA and RfGD-PDLLA (data not shown).

The results for surface energy and adhesion tension of water are presented in Table I. The determination of surface energy was based on the Owens and Wendt method[28] and performed using water and diiodomethane test liquids. In parallel, adhesion tension was also calculated as an alternative indicator of surface energy. RfGD-PDLLA films, when compared with control PDLLA films, showed significantly higher surface energy and water adhesion tension values. PDLLA surface energy significantly increased from

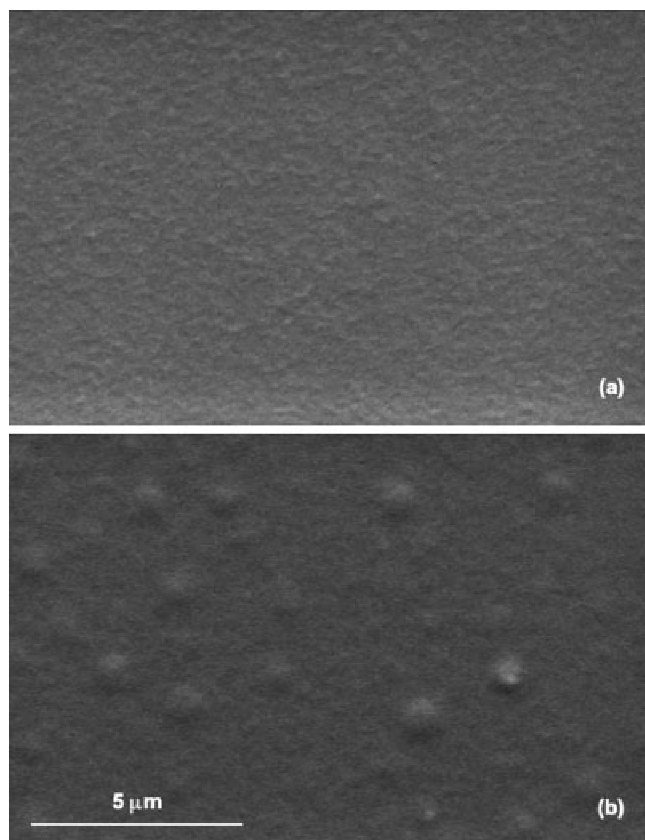

Figure 2. SEM characterization of surface morphology of PDLLA films: (a) before treatment; (b) after plasma treatment.

42.7 ± 2.3 dyne/cm to 50.0 ± 1.8 dyne/cm; the adhesion tension of water increased ~19 mN/m (from 18.3 ± 3.2 mN/m to 37.2 ± 1.8 mN/m). These results suggest the incorporation of polar groups as a consequence of oxygen plasma surface treatment.

SEM was used as a qualitative tool to detect any changes in morphology introduced by the plasma treatment (Figure 2). The control surface (nontreated PDLLA) exhibited a uniform texture [Figure 2(a)], while treated surfaces presented small and irregularly distributed protrusions of less than 1 μm diameter. Although these features were sparsely distributed, it is clear that oxygen plasma treatment can modify the microtopography of PDLLA films.

XPS was used for more detailed chemical analysis of the surface.[34] During the RfGD treatment the active plasma species attack the polymer surface resulting in the incorporation of additional carbonyl, carboxyl or hydroxyl functional groups.[35–38] XPS retrieves information on the nanometer scale, more specifically with up to a depth close to 50 Å.[33] XPS analysis results for treated and nontreated PDLLA films are shown in Table II. Following plasma surface modification there was a significant increase in the C—O—O bonds while the C—H and C=O functional groups decreased ($p < 0.05$). Also the total oxygen content exhibited a 4% increase, in contrast to carbon content, which decreased ~4%.

In this study FTIR-ATR was performed on nontreated and plasma-treated PDLLA films to detect any changes in chemical composition. Analysis of the spectra did not suggest treatment-driven chemical changes either in the aliphatic, carbonyl or asymmetric stretching regions[39,40] (data not shown). This may be because in contrast to surface sensitive methodologies such as contact angle or XPS, the FTIR-ATR collects spectra from a depth up to 100 nm below the surface.[33] Thus changes limited to the top few atomic layers (as detected by XPS) may become indistinguishable.

Protein Adsorption onto PDLLA- and RfGD-Treated PDLLA

Several surface properties can influence protein adsorption, such as morphology, chemistry, and hydrophobicity.[41] In this study, the potential of oxygen-based RfGD treatment to increase protein adsorption onto PDLLA was assessed. The degree of protein adsorption was carried out using an indirect method by coupling a solution depletion technique and a colorimetric protein (BCA) assay as described earlier. As shown in Figure 3, after RfGD treatment PDLLA surfaces adsorbed a significantly higher percentage of BSA and FN. The adsorption increased ~6 and 15% for albumin and fibronectin, respectively, which corresponds to 5.83 μg/mL of BSA and 15.3 μg/mL of FN. In contrast, FBS did not show any preference for either surface. The results for VN were inconclusive.

Behavior of Bone-Related Cells on PDLLA Films

The attachment and proliferation of FRC cells onto RfGD modified PDLLA was compared with the control TCPS and nontreated PDLLA surfaces. Cells were seeded on the surfaces for 3 h, and 7, 9, and 14 days followed by trypsinization and subsequent cell counting. Figure 4 show the results of cell numbers for the different post-seeding time points. The results indicate that the number of FRC cells on PDLLA was similar to the one observed for plasma modified surfaces. In contrast, BSA and VN had a positive

TABLE II. XPS Analysis Data: Atomic Percentages of Elements and Functional Groups for the Untreated and Oxygen RfGD-Modified PDLLA

	O1 (%)	O2 (%)	N 1s (%)	C—H (%)	C=O (%)	C—O—O (%)	Total O (%)	Total C (%)
PDLLA	14.4 ± 0.2	17.4 ± 0.3	1.2 ± 0.1	23.9 ± 0.5	23.2 ± 0.5	19.9 ± 0.1	31.8 ± 0.5	67.0 ± 1.1
RfGD-PDLLA	18.7 ± 0.5[a]	17.0 ± 0.3	1.1 ± 0.1	20.7 ± 0.2[a]	20.6 ± 0.1[a]	21.7 ± 0.4[a]	35.8 ± 0.8[a]	63.1 ± 0.7[a]

[a] Statistically different from PDLLA (t test; Bi-tail; $p < 0.05$; $n > 3$).

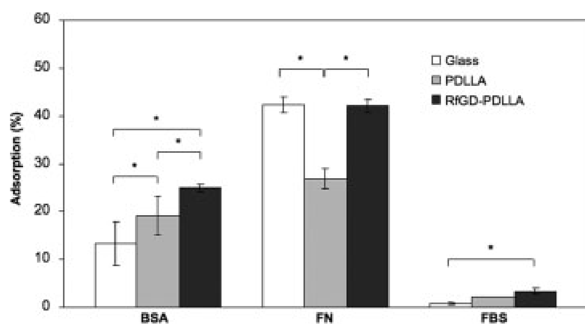

Figure 3. Percentage of adsorbed proteins, BSA, FN and FBS on PDLLA nontreated and treated by oxygen based RfGD. *Statistically different from PDLLA (*t* test; Bi-tail; $p < 0.05$; $n > 3$).

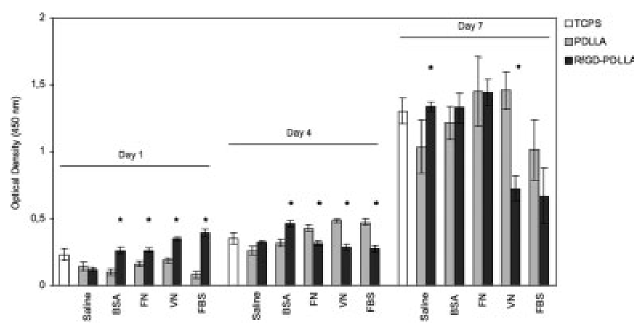

Figure 5. MG63 cells density for 1, 4, and 7 days of culture on control PDLLA and RfGD treated PDLLA precoated with proteins. *Statistically different from PDLLA (*t* test; Bi-tail; $p < 0.05$; $n > 3$).

influence on FRC cell attachment to gas plasma treated films as shown by the significantly higher cell numbers at 3 h (Figure 4). This can be attributed to the combination of plasma treatment and preincubation of the films with proteins. By day 14, however, the RfGD treated films with preincubated with FN showed a higher average cell number compared with the nontreated PDLLA. Thus, although BSA and VN appeared to cause an increase in cell attachment this effect did not translate into an advantage by day 14. Furthermore, cell proliferation was similar at days 7 to 14 in both controls and protein-exposed surfaces. Interestingly, this is the time when an increase in alkaline phosphatase expression has been reported previously.[42] Studies on gene expression and cell differentiation could be helpful for further understanding of the observed cell behavior.

In the study with MG63 osteoblast-like cells, the relative proportions of BSA, FN, and VN found in human blood were used and FBS was diluted to 1%. WST-1 mitochondria assay was performed for measuring the viability of MG63 cells after 1, 4, and 7 days of culture, taking into account intact mitochondrial mechanisms, consistent cellular activation and similar ECM interactions.[43]

Cell attachment and proliferation results for MG63 cells are shown in Figure 5. The test films performed as well as the culture standard TCPS. At day 1, there is an increase in cell attachment on RfGD treated PDLLA films precoated with BSA, FN, VN, FBS. However, by day 4 these trends were reversed except for BSA. By day 7 there was significant difference in cell numbers between the treated and nontreated PDLLA films was for the case of VN where the nontreated surfaces showed more cells. For days 4 and 7 of culture, there was a reversal in the relative density (OD) for treated and nontreated PDLLA films with preadsorbed VN and FBS compared to day 1. Also, as can be observed in Figure 5, only gas plasma treatment followed by saline pretreatment increased MG63 cell attachment by day 7.

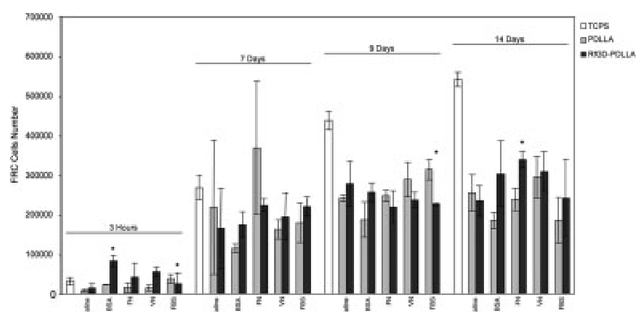

Figure 4. FRC cells attachment and proliferation after 3 h, 7, 9, and 14 days of culture on PDLLA and RfGD treated PDLLA films, previously incubated with different protein systems. *Statistically different from PDLLA (*t* test; Bi-tail; $p < 0.05$; $n > 3$).

DISCUSSION

The herein presented study aimed at assessing the influence of oxygen radio frequency glow discharge on the surface and bulk properties of poly(D,L-lactic acid) and the effect of this surface modification on both biomolecules adsorption and bone cell behavior. The main purpose of these experiments was to determine whether oxygen-based RfGD could improve the adsorption of various proteins, as BSA, FN, VN, and complex protein solutions as FBS and ultimately model cell attachment and proliferation of MG63 and FRC cells.

The surfaces were characterized by evaluating different key parameters such as the surface contact angle, surface energy and adhesion tension of the water. Oxygen-based RfGD treatment on PDLLA films was shown to significantly decrease surface contact angle to $\sim 60^\circ$ [Figure 1(b)], thus indicating an increase in the hydrophilicity of the RfGD-PDLLA surface. Furthermore, surface energy and the adhesion tension of water significantly increased to ~50.0 dyne/cm and 37 m*N*/m, respectively. These results suggest the incorporation of polar groups as a consequence of oxygen plasma surface treatment. Multiple studies in the past have shown that oxygen plasma exposure can render higher hydrophilicity on polymer surfaces.[36,37] The changes in wettability are generally due to effects of oxidation, unsaturation, electrostatic charges or surface morphology.[35,38] Thus, in this study we used a variety of techniques to further interrogate the test PDLLA surfaces. SEM analysis of PDLLA films indicated that RfGD introduced

some surface morphological changes. More precisely, changes in microtopography were evidenced by the increase in surface roughness. This factor has been related to the improve of hydrophilicity since surface microfeatures are known to affect wettability.[3] Chemical changes showed an increase in total oxygen atomic percentage ascribed to an increase in the oxygen-containing functional group C—O—O. The FTIR analysis, compared with the XPS results, suggests that the effects of the oxygen plasma surface modification were limited to the surface and did not affect the bulk properties of the material.

The potential of surface properties in influencing protein adsorption, such as morphology, chemistry and hydrophobicity was been evaluated.[41] In this study, the capacity of oxygen-based RfGD treatment to increase protein adsorption onto PDLLA was assessed. The degree of protein adsorption was carried out using an indirect method by coupling a solution depletion technique and a colorimetric protein (BCA) assay as described earlier. The amounts of adsorbed proteins in single systems (BSA, FN) were shown to increase after plasma treatment ($p < 0.05$), which modified PDLLA wettability, surface energy, and adhesion tension of water as discussed earlier (see Figure 1 and Table I). The relation between protein adsorption and contact angle or surface energy is controversial in the literature; it has been shown that decreasing surface hydrophilicity leads to lower protein adsorption[44] and, in contrast, it has also been reported that increasing surface energy leads to lower adsorption.[45] It must be stated that in this study, RfGD of PDLLA films did not result in extremely high or low contact angle or surface energy. According to Ikada,[46] intermediate values seem to be the most favorable for cell adhesion. The increase in O-containing functional group C—O—O and the increase in total O% of treated over nontreated samples as determined by XPS may promote protein-surface interactions. The introduction of oxygen functionalities creates sites for binding proteins by polar interactions or hydrogen bonding. Also, the simultaneous increase in surface micro-heterogeneity may play a role in the increased protein adsorption. However, at the same time, the degree of protein adsorption from multi-protein solutions was not affected by the change in surface properties. This could perhaps be explained by protein competition and the resulting protein conformations taking place on the surface. These factors could be further explored using other approaches, such as extrinsic fluorescent probes.

MG63 osteoblast-like cells and primary cultures of FRC cells were used to assess both the effect of RfGD treatment and protein adsorption on cell attachment and proliferation.

In the absence of previously adsorbed proteins, neither the attachment of MG63 nor that of FRC cells showed significant changes resulting from the treatment. Also, the proliferation of FRC cells up to 14 days was not affected by the RfGD treatment. In contrast, proliferative rates of MG63 osteoblast-like cells were higher for plasma treated PDLLA surfaces, showing a direct effect of the oxygen based plasma technique.

CONCLUSION

The surface modification technique selected for this study, oxygen-based RfGD treatment, was shown to functionalize/activate the surface of PDLLA films without affecting the bulk properties. After treatment under the described conditions, PDLLA films exhibited presented increased wettability, surface energy, and water adhesion tension. Chemically, changes showed an increase in total oxygen atomic percentage ascribed to an increase in the oxygen-containing functional group C—O—O. Moreover, RfGD introduced some surface morphological changes. Regarding protein adsorption studies, oxygen gas plasma treatment of PDLLA films was shown to improve BSA and FN adsorption from single protein solutions. On the other hand, adsorption from complex protein solutions (e.g. FBS) was unaffected by the material treatment. Thus, oxygen RfGD treatment resulted in PDLLA surfaces with preferred adsorption characteristics.

In the absence of preadsorbed proteins, neither MG63 nor FRC cells could distinguish between treated and untreated surfaces. However, MG63 osteoblast-like cells showed higher proliferation rates for plasma treated PDLLA surfaces, indicating a direct effect of the oxygen based plasma technique. In turn, gas plasma treatment, by influencing protein adsorption on the surfaces, was shown to affect cell response to the surfaces. The effect on the treatment over the cultured cells was only observed by combining gas plasma modification of the surface with the protein adsorption, thus indicating a crucial role for adsorbed proteins in mediating the response of osteogenic cells to the RfGD-treated PDLLA surface.

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